probes being capable of hybridizing to said amplification product in a contiguous manner to form a detection product;

Please add new claims 40-44, as follows:

40. The method of claim 1 wherein said amplification probes are approximately the same length and contain about 2 to 30 nucleotides.

41. The method of claim 14 wherein said amplification probes are approximately the same length and contain about 2 to 30 nucleotides.

42. The reagent of claim 19 wherein said amplification probes are approximately the same length and contain about 2 to 30 nucleotides.

43. The kti of claim 21 wherein said amplification probes are approximately the same length and contain about 2 to 30 nucleotides.

REMARKS

I. Description of the Present Invention

The present invention relates to a two-part method for the detection of nucleic acid target sequences, wherein a detection procedure may be employed in combination with an amplification procedure to achieve the detection of low levels of target in a test sample. Amplification is accomplished by using a denatured amplification sequence of the target as a template for the contiguous hybridization of a plurality of denatured pairs of complementary amplification probes. Ligation of the contiguously hybridized probes generates amplification product which is then be denatured from the amplification sequence, releasing additional template (denatured amplification product) to guide the contiguous hybridization of

amplification probes in subsequent cycles of the amplification reaction. This enables the exponential accumulation of amplification product.

Where three or more pairs of amplification probes are employed, appropriately designed detection probes can be used to distinguish correctly assembled amplification product from incorrectly assembled spurious amplification by-product. This type of spurious by-product is formed in solution without benefit of template sequence and, therefore, is not indicative of the presence of target in the test sample. If the presence of the spurious by-product is not minimized or distinguished in some way from the desired amplification product, the by-product creates serious background problems, negatively impacting sensitivity.

At least two detection probes are used in the present method, with each detection probe being complementary to a portion of each of two adjacently situated amplification probe segments of the amplification product. The correctly assembled amplification product serves as a template for the contiguous hybridization of the detection probes to form a detection product. The detection product can then be joined to form a ligated detection product in a manner similar to the ligation of contiguously hybridized amplification probes during amplification. Where the detection probes are joined, the resulting ligated detection product can, for example, be separated by polyacrylamide gel electrophoresis (PAGE).

II. 35 USC §112 Objections

The Examiner has set forth numerous objections to the specification and has rejected all of claims 1-39 under 35 USC §112 for failure to provide an enabling disclosure. Applicants, however, contend that the 8 working examples contained in the present specification, disclose and enable a variety of working formats of the present invention.

The Examiner has objected to the specification and rejected claims 3, 11, 17, and 39 under 35 USC §112, first paragraph, because of Applicants' suggestion, at page 15 of the specification, that, where heat denaturation is used to separate ligated amplification product from template sequence, it is preferred to use a thermostable ligase. According to the Examiner:

> Because heat denaturation is required in all instant examples, thermostable enzyme is essential to the claimed invention. The enzyme must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public....The specification does not disclose a repeatable process to obtain the enzyme and it is not apparent if the enzyme is readily available to the public.

The examples of the present application demonstrate the use of two different sources of the non-thermostable enzyme, *E. coli* ligase. These two different sources of enzyme are similar, but are different with respect to both purity and activity. All enzymes differ with respect to purity and activity, both of which are reported, as it is based upon these factors that assay adjustments are made. Applicants correctly noted, in their specification, that a thermostable ligase would be preferred for use in connection with the amplification method of the present invention, because a thermostable enzyme would eliminate the need for the addition of fresh enzyme following each heat denaturation step of the amplification procedure. In carrying out the experiments which are reported in Examples 1-8 of the present specification, Applicants employed ligases which were commercially available at the time the present application was filed. There is no basis to suggest that Applicants' invention, as presently claimed, is not enabled simply because Applicants have noted that the commercial availability of a particular enzyme would improve performance of their method.

The Examiner' rejection of claims 1-39 on the basis of the claims being limited to *E. coli* DNA ligase is likewise unfounded. It is the Examiner's position that:

> Only the use of *E. coli* DNA ligase has been disclosed as a means of ligating hybridized amplification probes and it would have required one of ordinary skill in the art an undue amount of experimentation to have found another enzyme that would have functioned in the assay as claimed.

The Examiner's reliance on M.P.E.P. §706.03(z) in support of this rejection is misplaced, because this section of the M.P.E.P. is directed to "cases involving chemicals and chemical compounds which differ radically in their properties". In these types of situations, "it must appear in an applicant's specification either by the enumeration of a sufficient number of the members of a group or by other appropriate language, that the chemicals or chemical combinations included in the claims are capable of accomplishing the desired result." In this regard, Applicants have previously cancelled broad generic claims which included other ligating means (i.e., not limited to ligase) such as chemical or photochemical ligation. It is inappropriate to now split the ligase species into further subspecies and contend that the present application is enabled only for the particular type(s) of ligase demonstrated in the examples. The activity of a ligase may be readily predicted. In fact, ligases are recognized in the art as being substantially equivalent, and are sold as such. (See catalog pages from New England Biolabs, attached hereto as Exhibit A.)

Thermostable ligases are simply another species of the ligase genus, and are now commercially available, as set forth in the Ampligase™ Thermostable DNA Ligase Kit specification sheet from Epicenter Technologies, a copy of which is attached hereto as Exhibit B.

The Examiner has rejected claims 1-39 under 35 USC §112, first paragraph, because, according to the Examiner, "the disclosure is enabling only for claims limited to a target nucleic acid sequence wherein the nucleotide sequence is known (or the putative sequence has been determined)." Applicants have previously argued that the identity of the nucleic acid sequence need not be known in all instances, because, for example, it would only be necessary to know the amino acid sequence of the final protein product encoded by a particular nucleic acid sequence in order to make effective use of the present invention. Nevertheless, Applicants have amended claims 1, 14, 19, and 21 to include the limitation that the amplification sequence be known.

Claims 1-39 have been rejected under 35 USC §112, first and second paragraphs, because the Examiner still contends that:

> Functional language should be recited in the claims regarding the probe length and also the length of one probe relative to the others as the limitations of the specification must be read into the claims. However, the specification does not teach probe length or relative probe length and is therefor not enabling for a plurality of denatured pairs of amplification probes. As probe number and respective probe lengths are critical to the hybridization reaction as it is both individual probe length, relative probe length, and excess concentration of probe that will "drive the reaction forward".

Applicants have previously argued that, as is known to those skilled in the art, probe length is not critical with respect to the reaction kinetics of the amplification/detection system of the present invention, but is critical only with respect to achieving specificity, in other words, distinguishing the target over the carrier DNA in a sample. Applicants have previously cited well known teachings in the hybridization assay art which prescribe the optimum size(s) required for the the overall length of Applicants' contiguously

hybridized amplification probes to distinguish the target over the carrier DNA.

Nevertheless, the Examiner states that:

> Applicants' remarks regarding probe length and excess concentration are not at all convincing because the arguments address a single probe length or overall length of the contiguously hybridized amplification probe and not relative lengths of the three or more individual pairs of probes prior to the ligation reaction, as claimed.

With respect to relative probe length, Applicants content that it is inherent from the present specification and claims that the probes need only be: (1) long enough to hybridize at the annealing temperature selected for the particular amplification scheme; and, (2) short enough to denature at the denaturation temperature selected for the particular amplification scheme. The particular relative probe lengths will be apparent to those skilled in the art, although, in most cases, it will be most convenient (but not necessary) for the probes to be approximately the same length. This is in fact taught in the examples where all amplification probes are the same length, i.e., 15 oligonucleotides.

III. 35 USC §103 Objections

The Examiner has rejected all of claims 1-39 of the present application under 35 USC §103 as being unpatentable over Carr and Whiteley *et al* in view of Mullis *et al.* The Examiner correctly characterizes Applicants' position that the Carr *et al* and Whiteley *et al* are directed to analytical methods which do not contemplate amplification. The Examiner contends, however, that:

> ...the test of obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would

suggest to those of ordinary skill in the art presumed to be familiar with them. (Emphasis added.)

The Examiner characterizes Applicants' position as a "failure" to consider the references together. The Examiner further states that:

> ...the rejection was made under 35 U.S.C 103, on the basis of what the combined teachings of the references would have suggested to one of ordinary skill in the relevant art, and not under 35 U.S.C. 102, on the basis of anticipation by any of the individual references....Again, one of ordinary skill in the art presumed to be familiar with the applied references would know that what was important was the formation of the complementary sequence and that whether one used short fragments (amplification probes) of DNA and a polymerase or short fragments of DNA and a ligase with or without a polymerase that the only thing of importance was the formation of a complementary strand which could be used in subsequent reactions as taught collectively by Mullis *et al*, Carr and Whiteley *et al.* (Emphasis added.)

Applicants did not "fail" to collectively consider the references cited by the Examiner. Applicants' position is that there is no teaching or suggestion in the prior art which would lead one to combine Mullis with Carr and/or Whiteley *et al* to achieve the amplification/detection scheme of the present invention. The Examiner appears to the use both the past and present tense interchangeably in assessing the obviousness standard of whether the invention, taken as a whole, would have been obvious to a person having ordinary skill in the art at the time the invention was made. Applicants suggest that this is perhaps indicative of the Examiner's continued reliance on hindsight reconstruction to support obviousness rejections of Applicants' claims.

The Examiner's own statements regarding the combined amplification/detection system of the present invention are in fact supportive of Applicants' position of nonobviousness:

> Examiner argues that the background caused by target independent ligation of non-hybridizing probes can defeat the entire purpose of the amplification procedure by masking results from samples at the sensitivities which require target amplification in the first place and is not prevented by the instant method in any way....By increasing the number of probes (3 or more) applicants simply statistically reduce the likelihood that correctly aligned amplification product in the absence of target sequence is formed. Their method does not, however, decrease the occurrence of spurious by-product. Thus applicants' method as claimed does not appear to address the problem of background caused by incorrectly aligned spurious blunt-end ligated amplification by-product. (Emphasis added.)

Applicants' method does in fact address the problem caused by incorrectly aligned spurious blunt-end ligated amplification by-product, and does so in an unobvious way. The Examiner appears, however, not to appreciate and/or believe that Applicants' system can work, as the Examiner goes on to state:

> Applicants state that their detection product serves as an indication of only the correctly assembled amplification product, nearly all of which is traceable to the presence of target.
> Examiner is confused by this statement because 1) any system employing a specific probe is capable of detecting correctly assembled target or amplification product as long as respective probes sequences are specific for the aligned fragment junctions, and 2) the statement seems to imply that one can quantitatively determine how much target existed in the original sample However, the argument appears to be faulty because if the correctly aligned spurious blunt-end ligated amplification by-product (low copy number) was formed early in the cycling and subsequent exponential amplification occurred there would be just as much if not more product produced from target-independent ligated by-product than from original target of interest. (Emphasis added.)

The Examiner's remarks are consistent with the teachings of the prior art which suggest that blunt ligation in a ligase-based amplification system must be addressed by reducing the amount of blunt-end ligated spurious amplification by-product formed. (See European Patent Application No. 320,308 Barringer *et al*, *Gene*, *89*, 117-122 (1990), and Kwoh *et al*, *Am. Biotech. Lab.*, *8*(13) 14-25 (1990), previously made of record.) In contrast, Applicants' method allows blunt-end by-product to form and instead discriminates the non-target-derived blunt-end ligated product from the desired amplification product. As a result, the detection product serves as an indication of only the correctly assembled amplification product, nearly all of which is traceable to the presence of target.

Applicants' argument is anything but faulty. It is Applicants' unique approach to addressing the blunt-ligation problem which enables Applicants to increase the number of pairs of probes to improve sensitivity in an assay. This advantage is clearly set forth in the specification at page 16, line 23 to page 17, line 13. Furthermore, during a May 3, 1990 interview, Applicants provided for the Examiner's review a series of autoradiogram photographs with graphic legends which demonstrate that, where the detection system of the present invention is employed, five pairs of amplification probes provide greater sensitivity than four pairs, which, in turn, provide greater sensitivity than three pairs. Applicants were informed by the Examiner that it was not necessary to produce evidence to support Applicants' position (which is set forth in the specification), because the Examiner is not allowed to question Applicants' position as stated in their specification or responses. However, the Examiner now contends that Applicants' argument is "faulty". In response to this contention, Applicants have produced these same photographs and graphic legends in the Declaration of Rodney M. Richards, which is filed concurrently herewith.

Applicants have amended independent claims 1 and 19 to include the limitation previously introduced into claims 14 and 21, requiring a minimum of at least three pairs amplification probes.

Thus, Applicants' invention, as presently claimed, corresponds to Applicants' unique and unobviousness combined amplification/detection system.

For the foregoing reasons, Applicants believe the present application is in condition for allowance, and an early notification to that effect is earnestly solicited.

October 7, 1991

Julia E. Abers

Julia E. Abers
Reg. No. 31,222
Amgen Inc.
Amgen Center
1840 Dehavilland Drive
Thousand Oaks, CA 91320-1789